Early Exposure to Bisphenol A and Lead: Effects on Metabolic Homeostasis and the Epigenome

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Children's Environmental Health and Disease Prevention Research Centers Meeting

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Presentation Overview

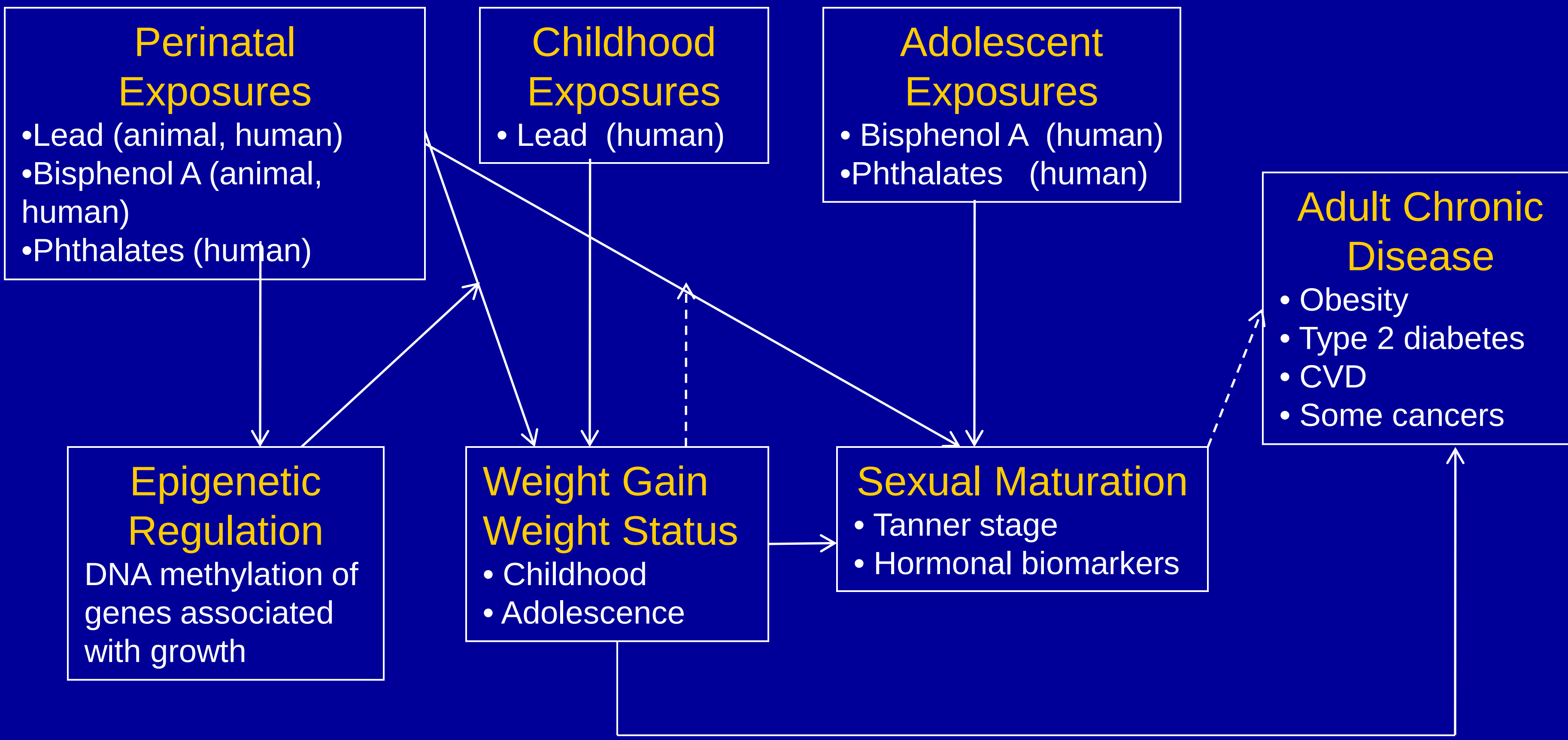
- Conceptual Framework of UM SPH P20 Center; Intro environmental and nutritional epigenetics

- **Example:** Early bisphenol A (BPA) exposure and metabolic homeostasis

- **Example:** Early lead (Pb) exposure, metabolic homeostasis and neuropathology

Conceptual Framework

Perinatal exposures, epigenetics, child obesity and sexual maturation



Center PI: Karen E. Peterson

Epigenetics in a Genetic Context

DNA (human): 3.2 billion bases (haploid), ~23,000 genes, 2 meters; A typical cell: 10-100 micro meters

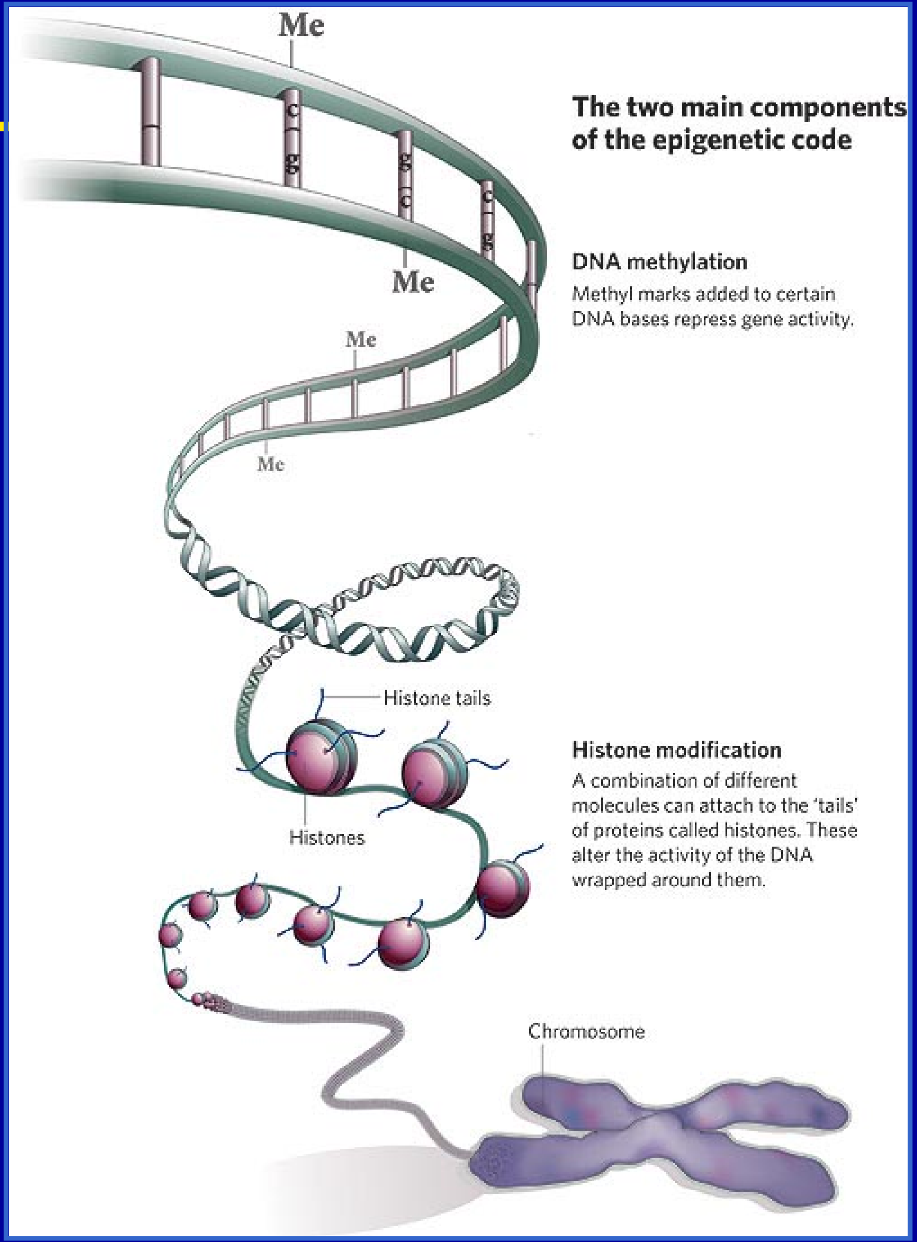
Epigenetic marks:

- DNA methylation
- Histone modifications

DNA is “packed.” ...But creates challenges and opportunities for regulation of gene transcription

Environmental epigenetics and the developmental origins of disease

Epigenetic plasticity may allow for pharmacological or nutritional intervention/prevention/treatment approaches

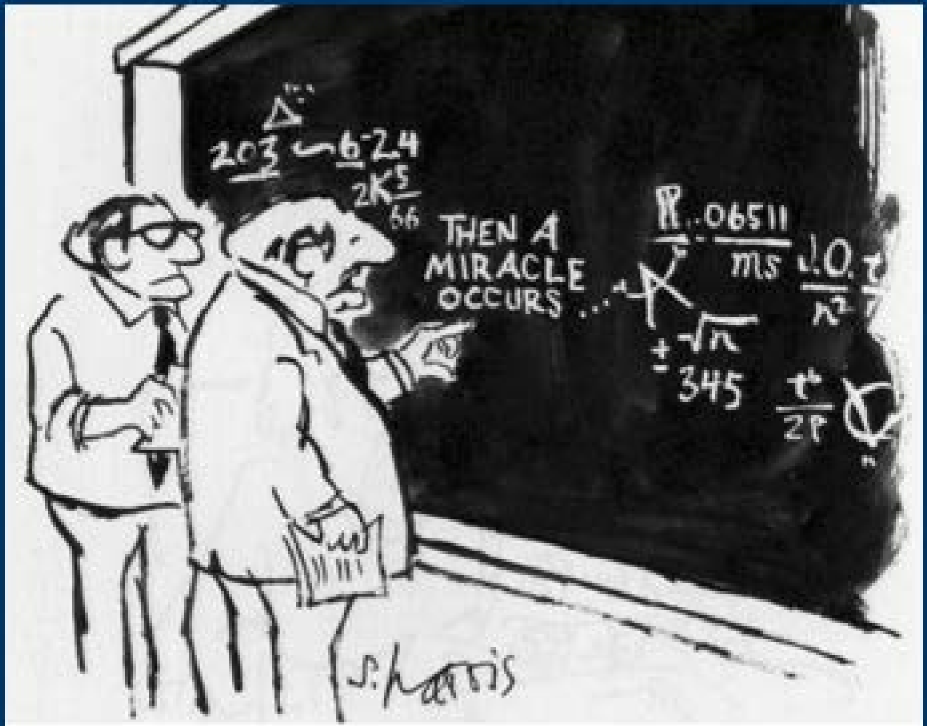


Why We Care: Early Origins of Disease

The Barker Hypothesis (1992)

Poor nutrition during gestation alters the development of an unborn child such that it will be prepared for survival in an environment in which resources are likely to be short, resulting in a **thrifty phenotype**.

However, often an **environmental mismatch** occurs**.** Those who develop in an affluent environment may be more prone to metabolic disorders, such as obesity and type II diabetes.



"I think you should be more explicit here in step two."

Miracle: Epigenetic Modifications

Epigenetic Susceptibility

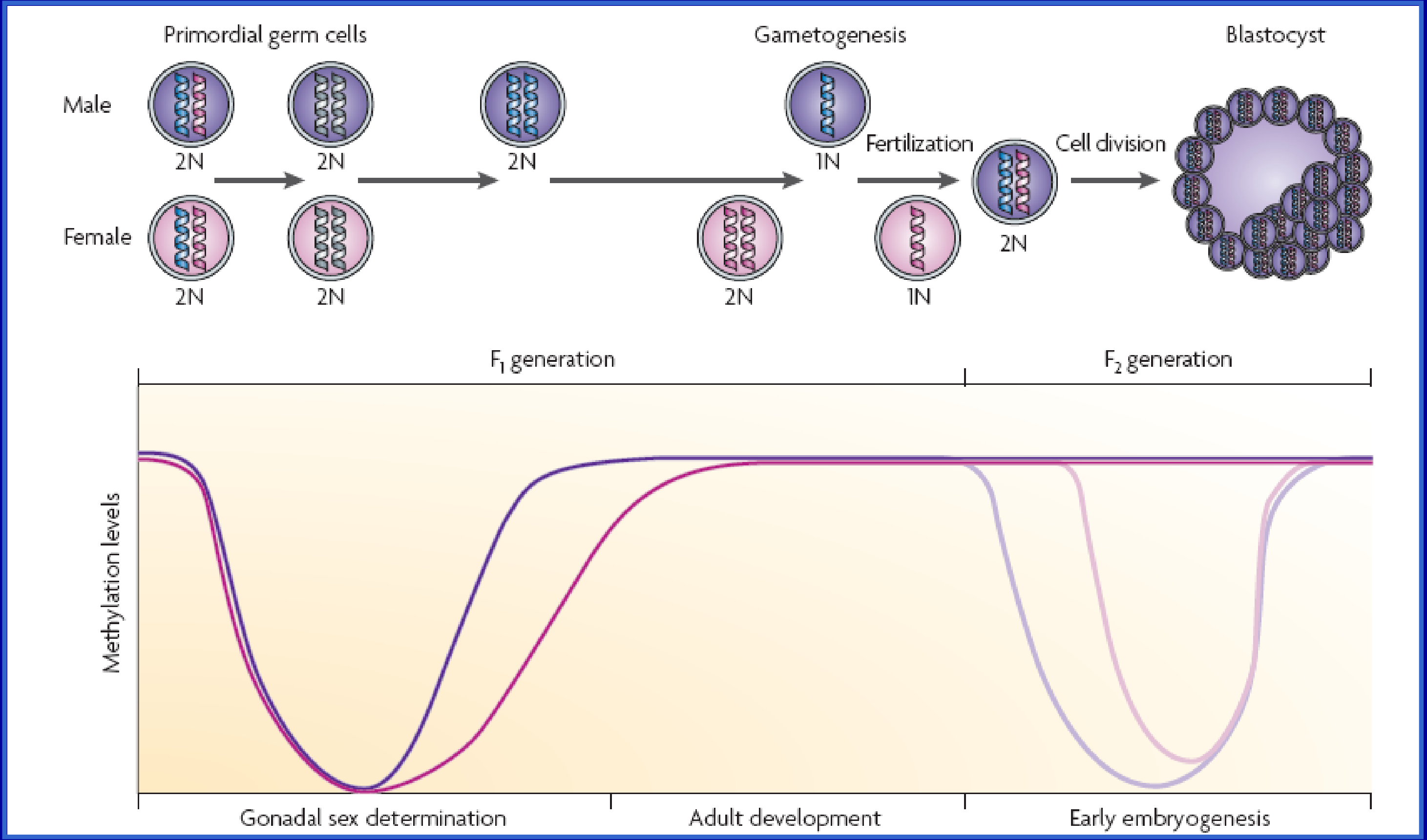
Gametogenesis

Pre-implantation stage of embryogenesis

Fetal and neonatal periods of development

Puberty

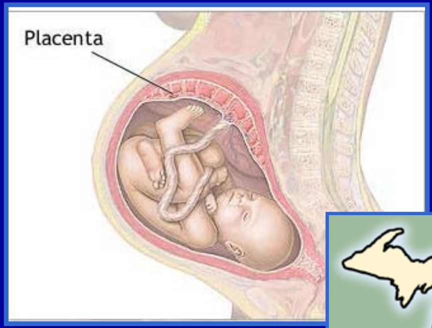
Old age



Jirtle and Skinner *Nature Reviews Genetics* 2007

Mouse to Human Experimental Approach

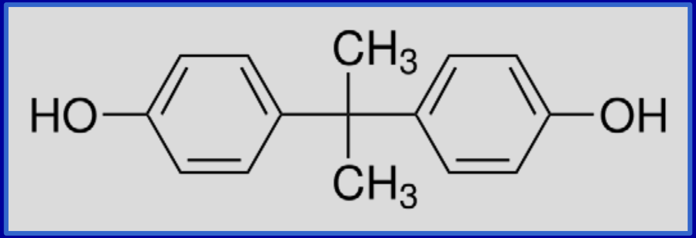
A^{vy}
Model(multiple doses)



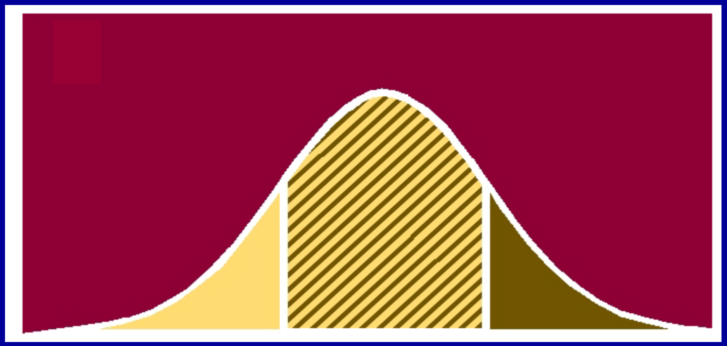
Human Clinical Samples

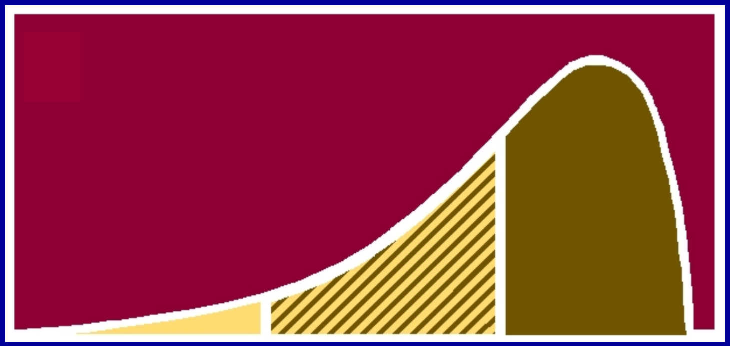
Population-based Cohorts

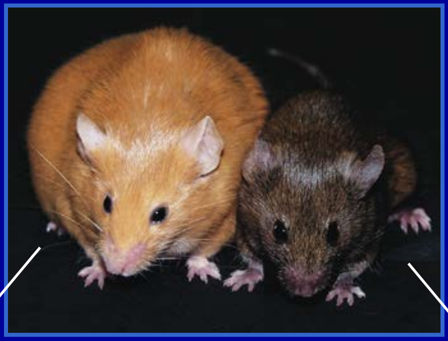
Perinatal Bisphenol A (BPA) Exposure, Epigenetics, and Metabolic Homeostasis



Viable Yellow Agouti Mouse Model: Epigenetic Biosensor







Methyl Donors

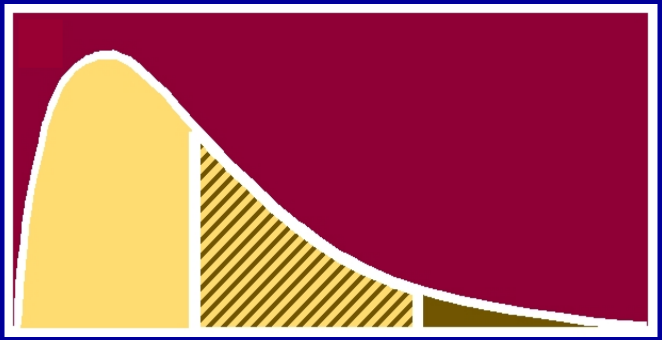
(Waterland *et al.* 2003)

DNA unmethylated	DNA methylated
Histone acetylation	H4K20 methylation
Ectopic expression	Little to no expression
Adult onset obesity	Lean

Genistein

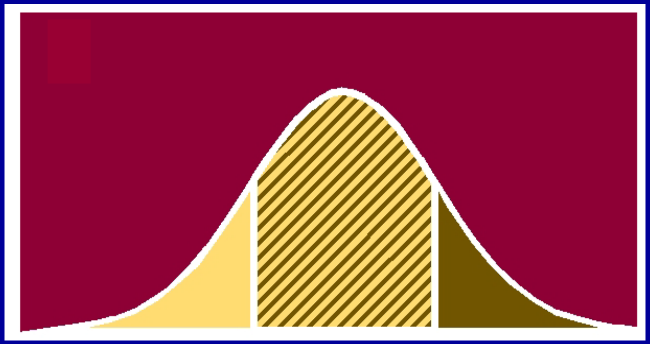
(Dolinoy *et al.*, Environ Health Perspect 2006)

Maternal Bisphenol A (BPA) Exposure



Bisphenol A

(50 mg BPA/kg diet)



Bisphenol A

(50 mg BPA/kg diet)

Maternal Nutritional Supplementation

Methyl Donors

Genistein

Dolinoy, et al. 2007 *PNAS*

Goals of Current Research

1) Expand dose-response assessment
2) Move from candidate gene driven to full epigenome technologies
3) Link epigenetically labile loci with biological pathways or phenotypes/health outcomes
4) Move from animal models to human clinical samples to human population approaches

(1) Moving from Single to Multiple Doses

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a/a non-agouti

A^{vy}/a agouti

2 Weeks Prior to Mating - 1 of 4 Diets:

1) AIN 93G Control
2) 50 mg BPA/kg Diet
3) 50 ug BPA/kg Diet
4) 50 ng BPA/kg Diet

50% *a/a* offspring

50% A^{vy}/a offspring

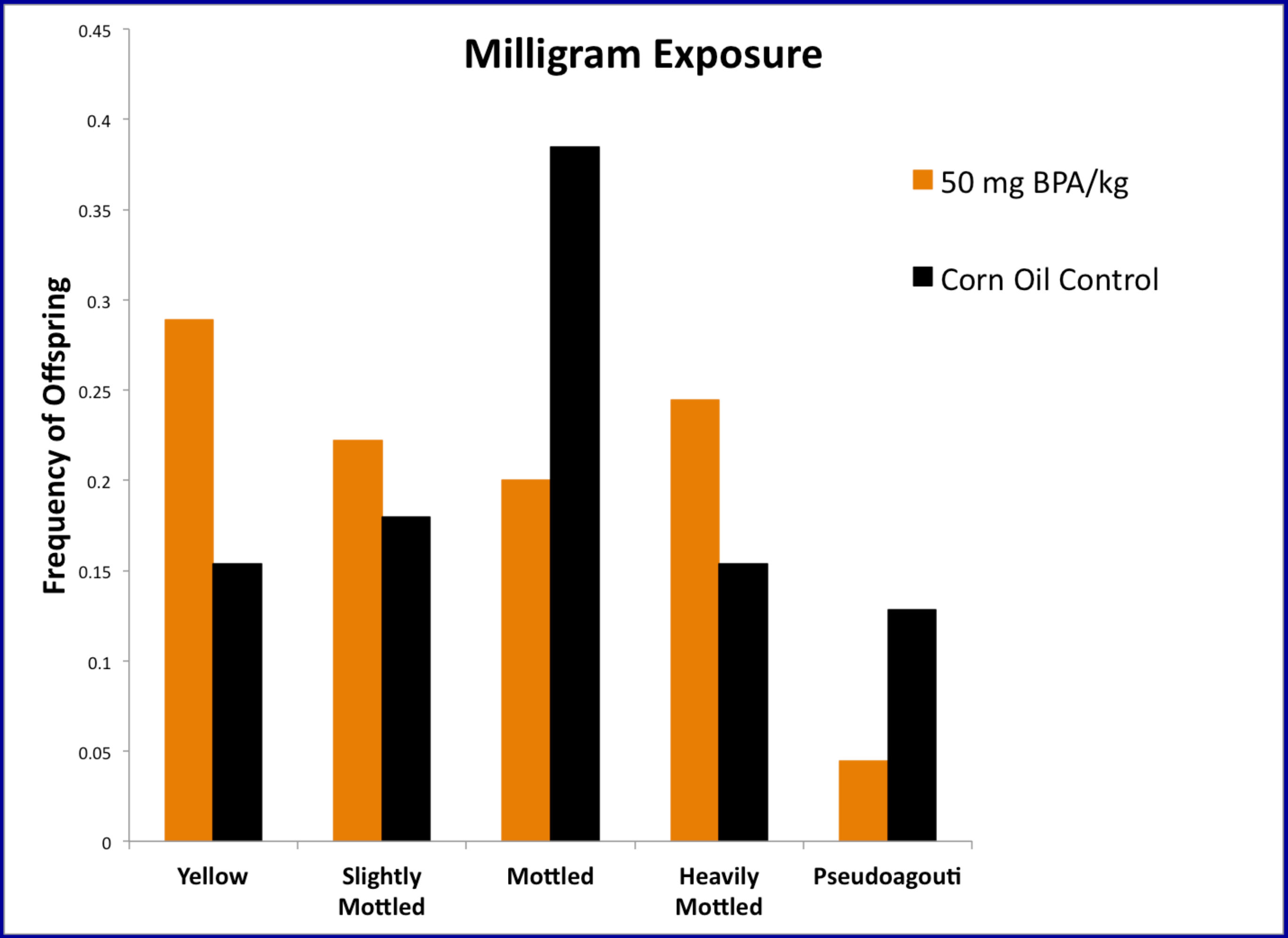
Environmentally Relevant Levels? Liver Tissue Levels in ng/g

Work in Progress! Collaboration with K. Kannan, Wadsworth Institute in Albany, NY

(Fetal samples from BDRL at Univ. Washington)

Nahar M., Anderson O. In Preparation

Dose Assessment - Coat Color Shift
Milligram (50 mg/kg diet) Dose Level

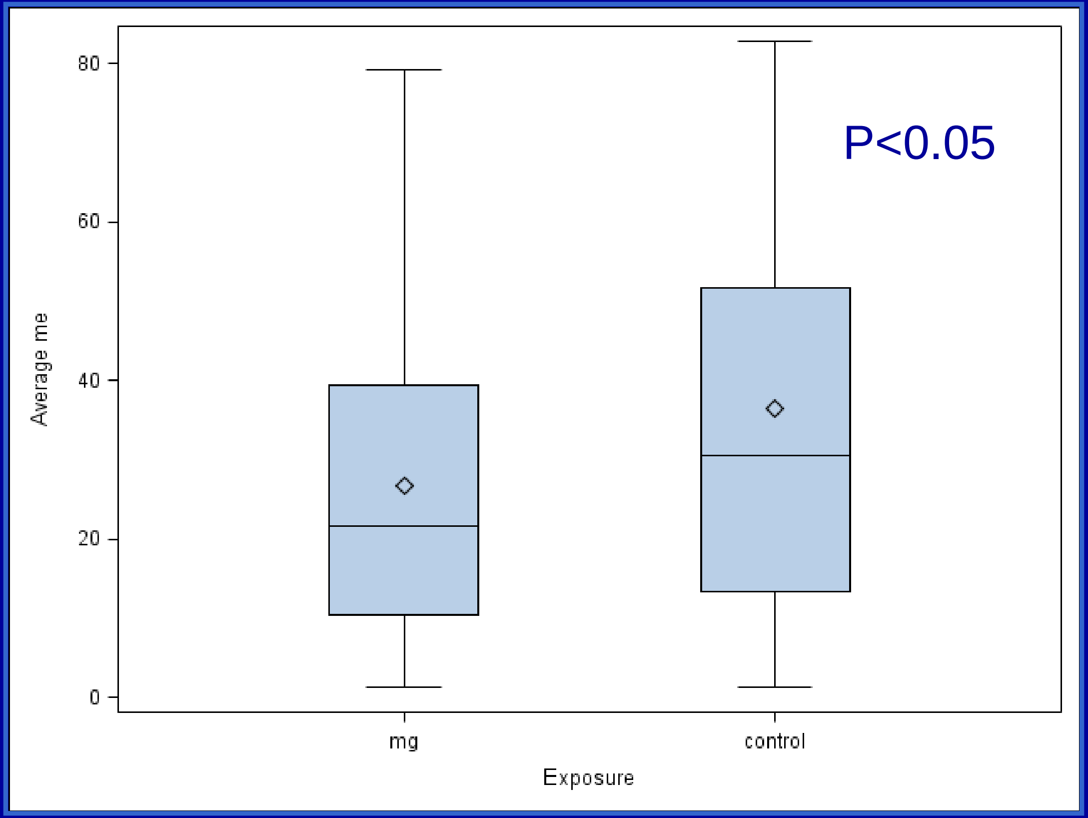


(p=0.006);

Mirrors 2007 *PNAS* findings

Anderson O. et al. In Press

A^{vy} Methylation Analysis:
Milligram (50 mg/kg diet) Dose Level



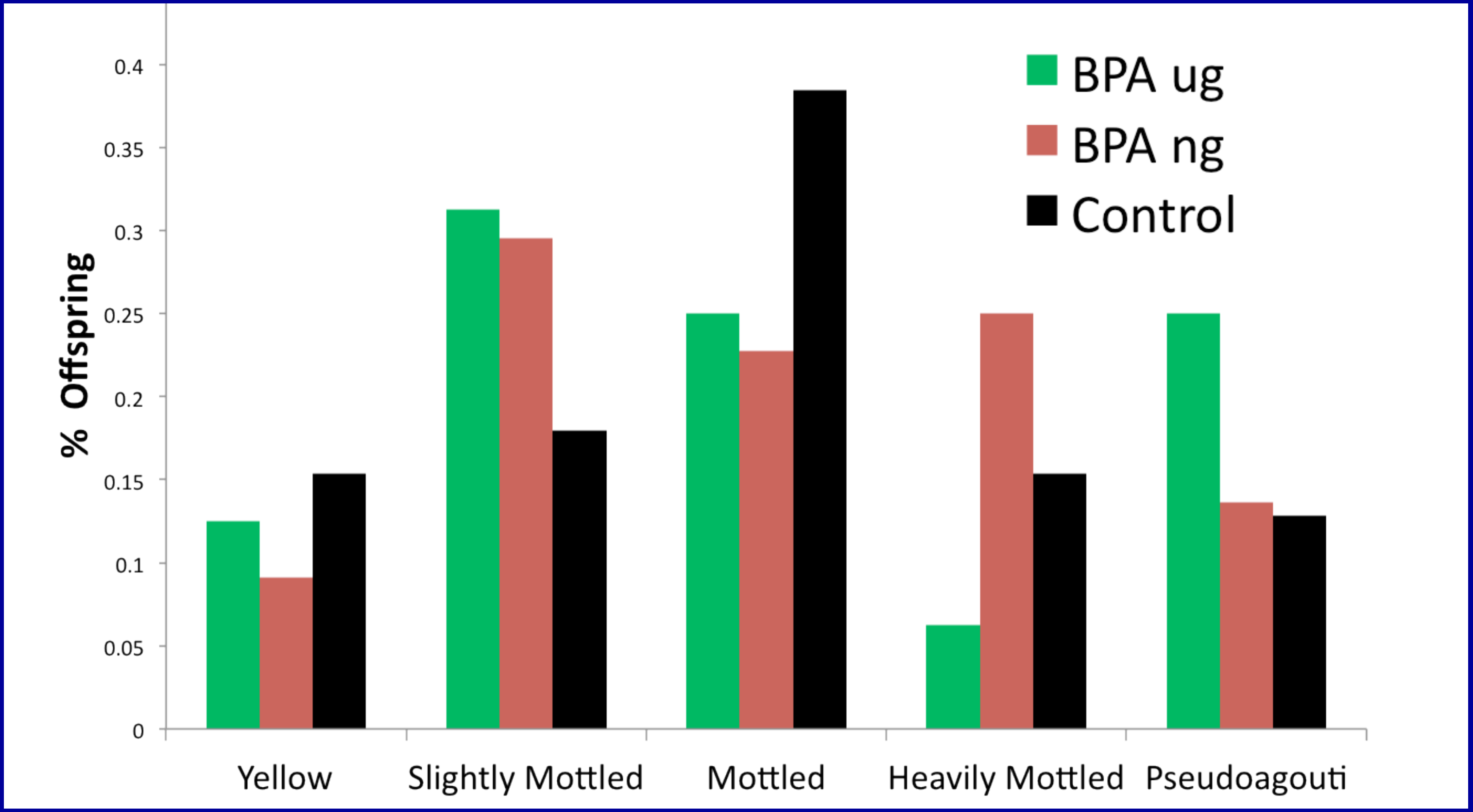
Exposure	Mean	Mean: *PNAS* 2007
mg	24.3	27
control	35.63	39

Anderson O. et al. In Press

Dose Assessment - Coat Color Shift

Microgram (50 ug BPA/kg diet)

Nanogram (50 ng BPA/kg diet)

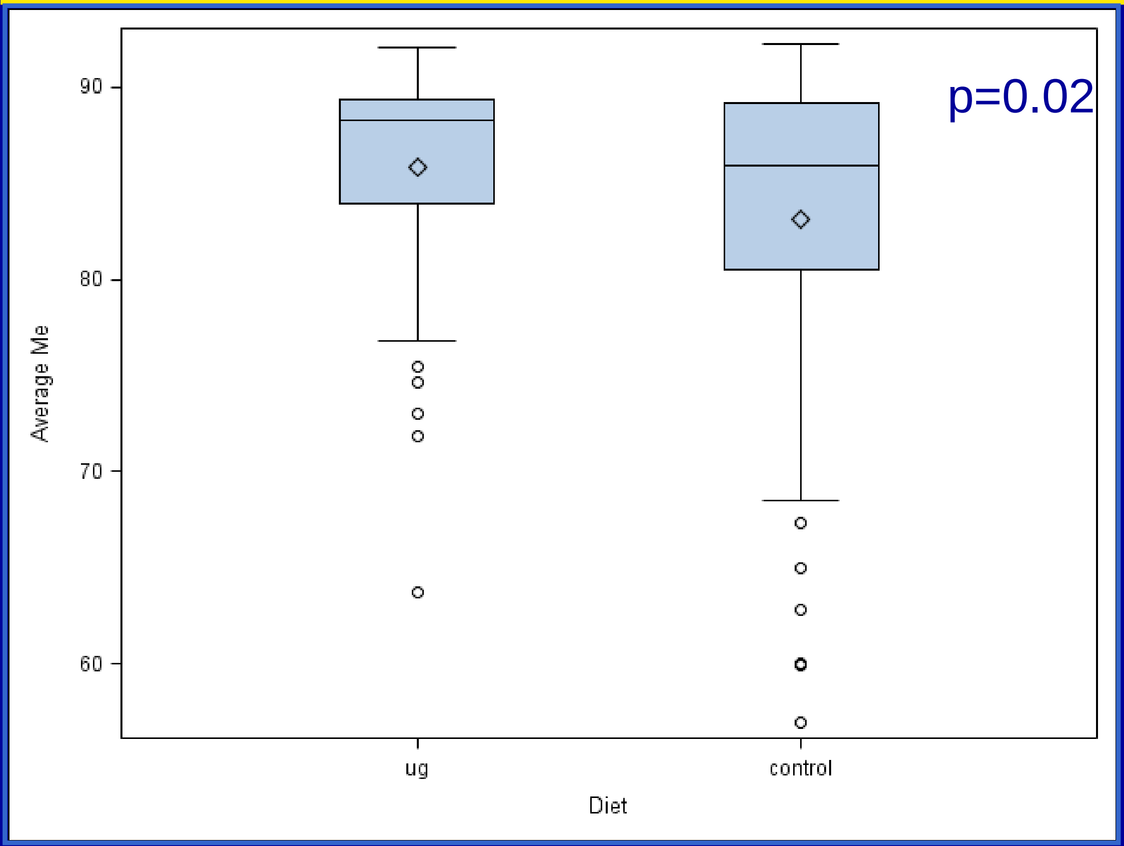


50 ug (p= 0.04)

50 ng (p=0.02)

Anderson O. et al. In Press

Cabp^{IAP} Methylation Analysis:
Microgram (50 ug BPA/kg diet) Dose Level

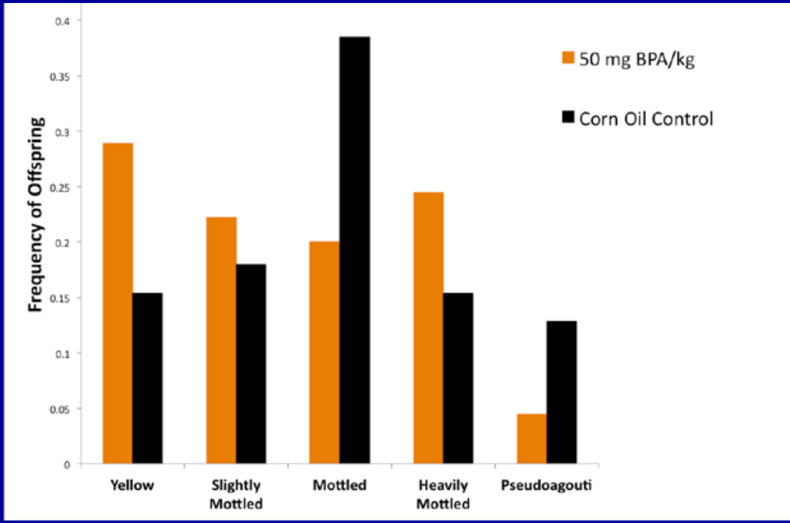


Diet	N	Mean	SD
ug	67	85.80	5.62
control	82	83.12	8.23

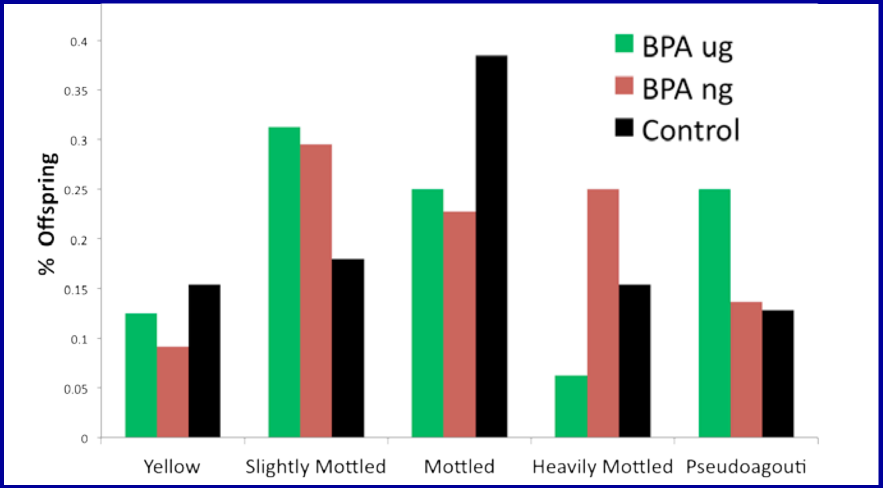
Anderson O. et al. In Press

A^{vy} locus as an Epigenetic Biomarker

Milligram Exposure



Microgram and Nanogram Exposure

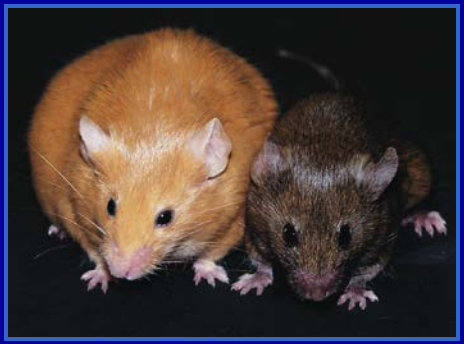


Unmethylated Methylated

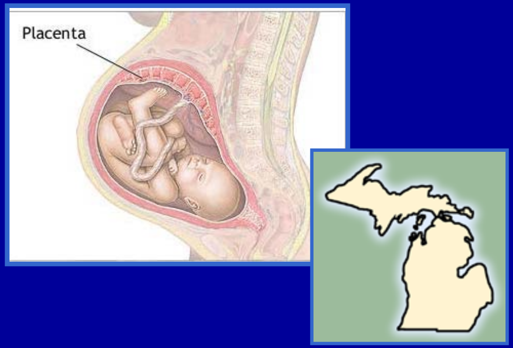
Unmethylated Methylated

Non-monotonic

(2) Moving from Candidate Gene to Whole Epigenome - Multi-Platform (Multi-Tissue) Approach



A^{vy} Model - Liver tissue plus blood



Human Clinical Samples – Fetal liver, placental tissues, cord blood

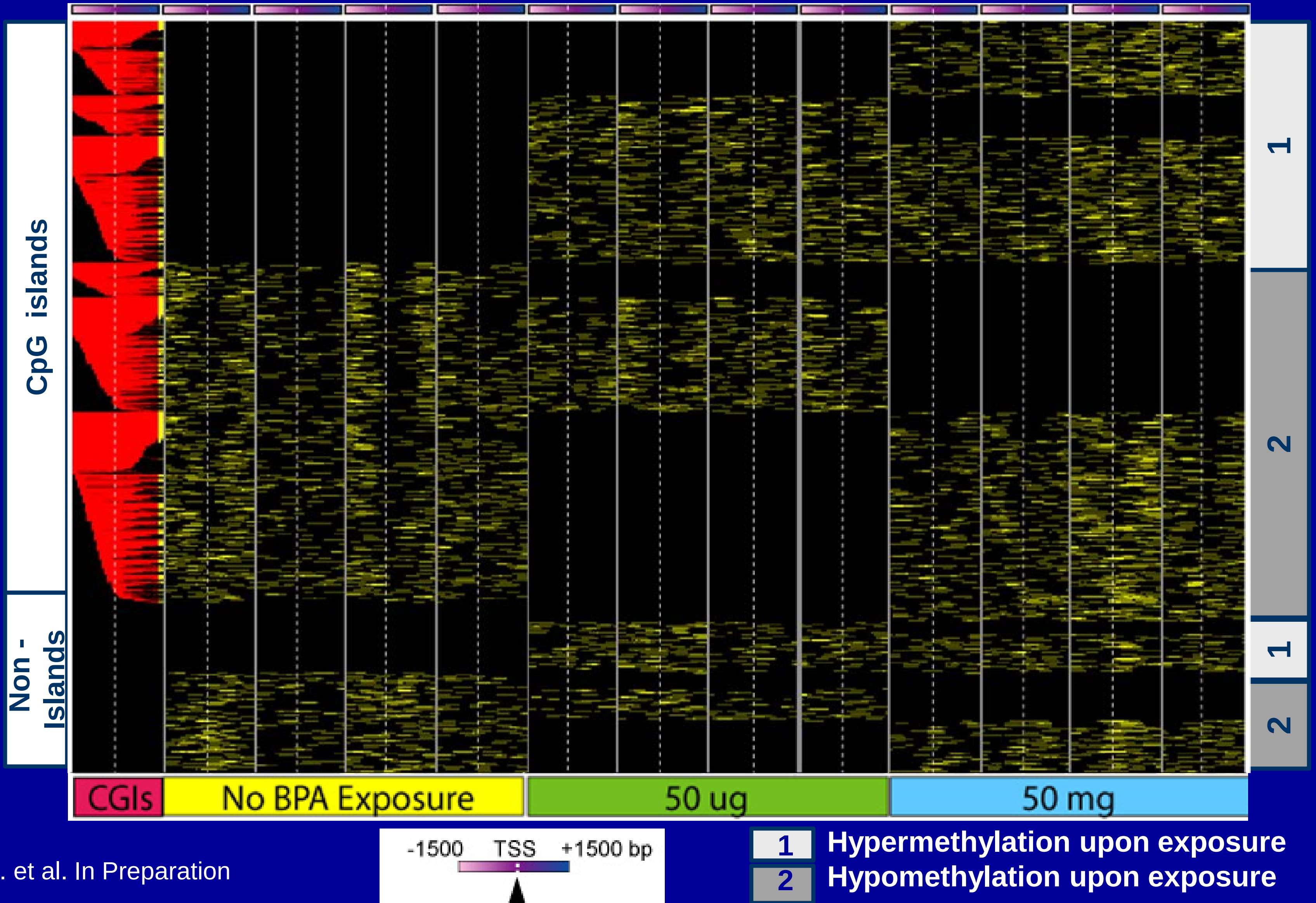
Perinatal Bisphenol A (BPA) Exposure

Methylation Deep Sequencing followed by validation with quantitative bisulfite sequencing

(+) Unbiased

(-) Expensive, complex bioinformatics

Differential Promoter Methylation by Dose



1 Hypermethylation upon exposure
2 Hypomethylation upon exposure

Kim J. et al. In Preparation

Pathway Enrichment Analysis

GOID	GO_term - Function	Corrected P-value
GO:0005488	binding	1.4528E-18
GO:0005515	protein binding	9.2311E-10
GO:0003824	catalytic activity	1.8317E-08
GO:0043169	cation binding	6.9366E-05
GO:0046872	metal ion binding	7.7010E-05
GO:0043167	ion binding	8.1632E-05
GO:0016787	hydrolase activity	1.0553E-03
GO:0008233	peptidase activity	1.3604E-02
GO:0016491	oxidoreductase activity	1.5233E-02
GO:0070011	peptidase activity, acting on L-amino acid peptides	1.5560E-02

Enriched in binding activity

GOID	GO_term - Process	Corrected P-value
GO:0009987	cellular process	3.0063E-18
GO:0008152	metabolic process	6.4477E-16
GO:0065007	biological regulation	4.2654E-10
GO:0044238	primary metabolic process	1.1912E-09
GO:0050789	regulation of biological process	1.8678E-09
GO:0044237	cellular metabolic process	3.9161E-08
GO:0050794	regulation of cellular process	1.7552E-07
GO:0050896	response to stimulus	1.0076E-06
GO:0043170	macromolecule metabolic process	2.8598E-06
GO:0019222	regulation of metabolic process	1.3345E-05

Enriched in metabolic processes

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(3) Linking Epigenetic Effects to Adverse Phenotype

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a/a non-agouti

A^{vy}/a agouti

2 Weeks Prior to Mating - 1 of 4 Diets:
1) AIN 93G Control
2) 50 mg BPA/kg Diet
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4) 50 ng BPA/kg Diet

50% *a/a* offspring

50% A^{vy}/a offspring

Life-Course analysis of phenotypes related to obesity/metabolic disorders/cancer

Life-Course Phenotyping (ongoing)

- D22 - Adiponectin and leptin
- D90 - Free fatty acids; oxidative stress markers (NIEHS BPA Supplement Award to V. Padmanabhan)
- 3, 6 and 9 months - Body composition; energy intake/expenditure; spontaneous activity
- 9 mo – Glucose tolerance test
- 10 months - Tissue collection; adiponectin & leptin levels; epigenomics (tiling arrays); tumor burden

Life-Course Activity Patterns Associated with Perinatal BPA Exposure

Results

- **No difference in food intake**
- Increased oxygen consumption and activity in female offspring
- Exposed females weigh less (with decreased fat mass) than the controls over each visit, but not statistically significant
- Female-specific results mirror Braun et al. findings in human population cohorts

Caveats

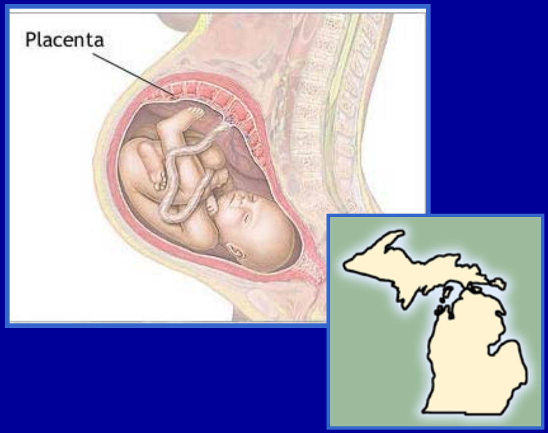
- Phytoestrogen free background diet
- Mice were not challenged with high-fat diet

CLAMS – Comprehensive Lab Animal Monitoring System

Future Directions

- Candidate gene methylation/Promoter tiling arrays

(4) Moving from Animals to Humans
Clinical and Population Samples



Human Clinical Samples

Population-based Cohorts

Bisphenol A (BPA) exposure

PI: Dana Dolinoy
Project: NIH-funded fetal tissue bank (Univ. of Washington)

PI: Vasantha Padmanabhan
Project: Maternal and term Cord Blood from UM Hospital

PI: Karen Peterson
Mexico City Birth Cohort (NIH/EPA Children's Env. Health Formative Center P20)

Pilot Project funded by UM NIEHS P30 Core Center
Collaborators: Amr Soliman, Laura Rozek
Project: Egyptian Girls

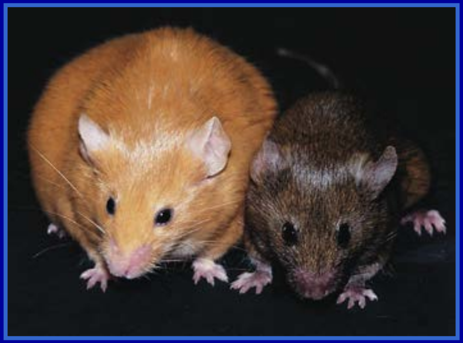
Early Life Exposures in Mexico to Environmental Toxicants (ELEMENT)

- ELEMENT is >15-year birth cohort comprised of mother-child pairs recruited from Mexico City during pregnancy and followed throughout childhood and adolescence.

- Biomarkers of exposure are available at various developmental time points (e.g., urinary BPA/phthalate measures; blood lead levels).
- Growth parameters and sexual maturation (tanner staging/hormones) are monitored overtime in the children.
- Epigenetic analyses is ongoing including methylation analysis of *LINE1* repetitive elements and, key growth genes and hormone receptors (*IGF2, H19, HSD11B2, PPARA, PPARG*) using DNA from birth and later time points.

- For P20 Target Sample Size = 200; Currently recruited ~100 pre-adolescent/early adolescent offspring.

Lead (Pb) DoHAD and Epigenetic Epidemiology



A^vy Model

ELEMENT Cohort

Perinatal Lead (Pb) Exposure, Epigenetics, and Metabolic Homeostasis

Leasure et al. report increased BW in 1 year old males following maternal Pb exposures with peak BLL ~10 ug/dL and ~25 ug/dL

Puzas et al. observe increased adipocyte differentiation in stem cells exposed to Pb

We expand to humans and lower doses in animal model with sophisticated measurements

Incorporate blood, fat, and brain concordance of DNA methylation and gene expression (animal model)

Perinatal Lead Exposure

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a/a non-agouti

A^{vy}/a agouti

2 Weeks Prior to Mating:

1) Control
2) 3 ppm (~peak BLL 2 ug/dL)
3) 27 ppm (~peak BLL 10 ug/dL)
4) 55 ppm (~peak BLL 25 ug/dL_

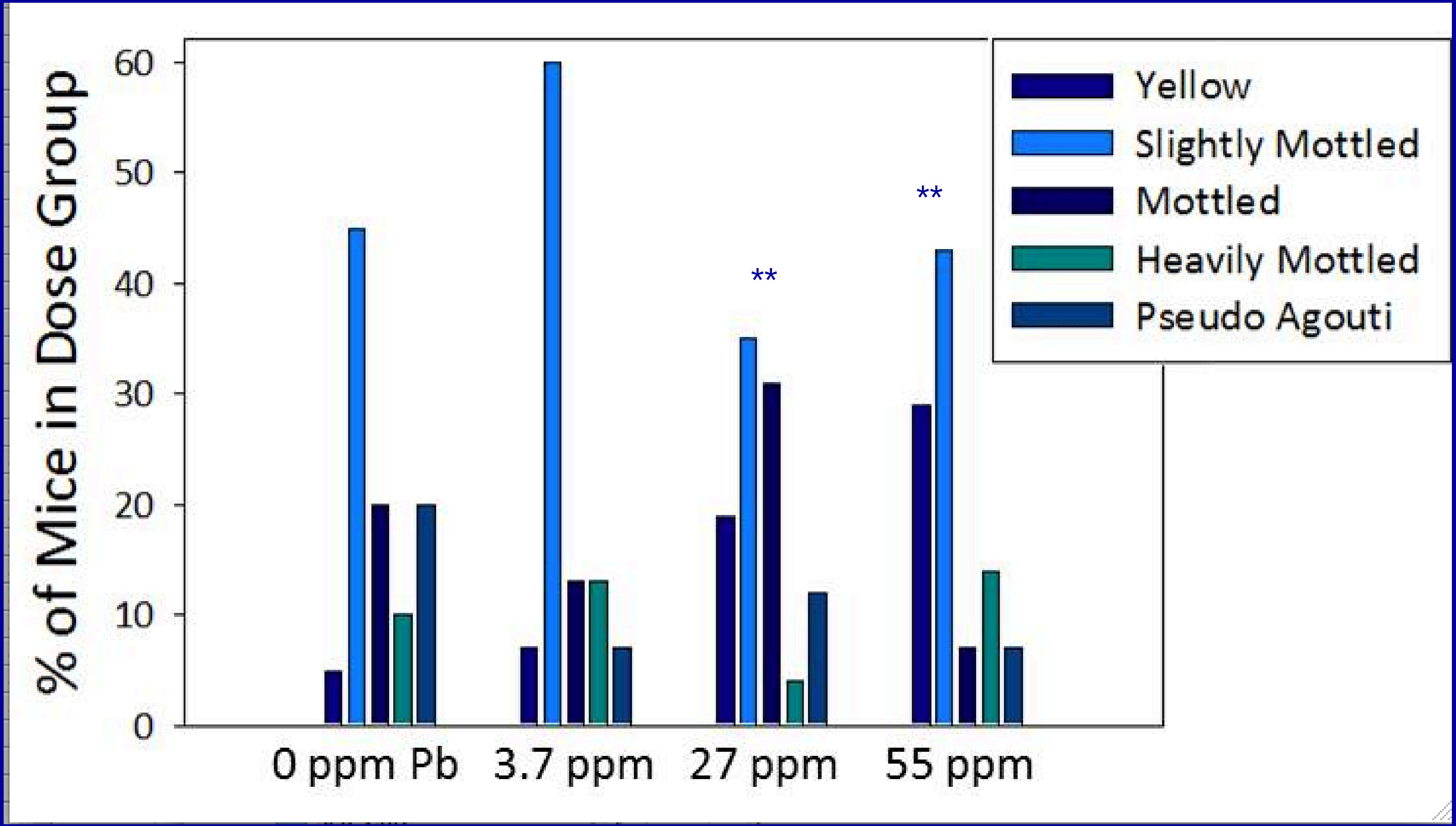
50% *a/a* offspring

Life-Course analysis of phenotypes related to obesity/metabolic disorders

50% *A^{vy}/a* offspring

Avy epigenetic biomarker

Preliminary Results:
Lead (Pb) and Coat Color Shifts



N = 6 to 8 litters per group

** Significant coat color shirts toward yellow are observed among offspring from the 27 ppm and 55 ppm Pb groups compared to controls (χ^2 p-value=0.009 and 0.006).

Perinatal Lead Exposure

♀  X  ♂

a/a non-agouti

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Conclusion and Future Direction

- Dose and full epigenome studies are crucial to deciphering the role of the environment on the epigenome

- Identification of epigenetically labile genes in the **Mouse and Human** **(and other model species)**

- Link epigenetically labile loci with biological pathways and phenotypes/human health outcomes

- DNA methylation in concert with other factors such as histone modifications and ncRNAs

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